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Blood-Squirting Variability in Horned Lizards (*Phrynosoma*)

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Variability within the genus *Phrynosoma* in the occurrence of ocular-sinus blood-squirting, reportedly a defense used in canid encounters, is reviewed from the literature. Six species have been reported to squirt blood, and seven species remain unreported. Five of the latter species were tested in dog trials; one exhibited blood-squirting (*Phrynosoma hernandesi*), one exhibited precursor behaviors but failed to squirt blood (*Phrynosoma ditmarsi*), and three yielded negative results (*Phrynosoma mcallii, Phrynosoma modestum,* and *Phrynosoma platyrhinos*). Instances of blood-squirting in response to human encounters were collected and largely support the negative results for the three species *P. mcallii, P. modestum,* and *P. platyrhinos.* A phylogeny of blood-squirting and nonblood-squirting species is presented with blood-squirting being plesiomorphic in the genus and the synapomorphic condition of nonsquirting species being restricted to a single clade of *P. mcallii-modestum-platyrhinos.* The possibility of *P. douglasii* independently evolving an autapomorphic condition remains unresolved. Dog trials with 40 adult *Phrynosoma cornutum* were conducted to determine influences of body size and sex on squirt frequency and blood mass expelled, as well as to examine aspects of the potential physiological cost of the defense. In 153 trials, 85% of all lizards squirted in at least one trial, 82% squirted in more than one trial, and two lizards squirted daily over the seven-day trial period. Initial body mass positively correlated with the total number of squirts/individual ($r^2 = 0.28$; $P < 0.001$) and the number of days a lizard continued squirting ($r^2 = 0.63$; $P < 0.01$). Number of squirts/individual/day declined over the seven-day trial period ($r^2 = 0.20$; $P < 0.05$). Cumulative mass loss for individual lizards attributable to blood-squirting averaged 0.7 ± 0.8 g (2.0 ± 2.0% body mass), with a high of 2.8 g (6.8% body mass). In addition, juvenile *P. cornutum* and *P. hernandesi* were shown to squirt blood in dog trials, illustrating the early developmental onset of the behavior.

BLOOD-SQUIRTING in the Texas horned lizard, *Phrynosoma cornutum* (Harlan), can be elicited by a canid (*Canis familiaris*) under a variety of environmental settings, including high or low temperatures, and day or night (Middendorf and Sherbrooke, 1992). The high frequency of blood-squirting in dog "predator" trials (70–100%) and the lack of a blood-squirting response to threats of other predators (e.g., roadrunners, *Geococcyx californianus* and grasshopper mice, *Onychomys torridus*; Sherbrooke 1990a, 1991), support the hypothesis that this stereotypic response is an antipredator defense specifically used with canid and possibly other mammalian predators.

With the accumulation of blood-squirting reports in horned lizards, remarkably distinctive lizards in terms of morphology and ecology (Pianka and Parker, 1975; Sherbrooke, 1981), the genus has been treated as if all species squirt blood (Stebbins, 1954; Smith and Brodie, 1982). This view persisted even though for most species blood-squirting behavior in response to encounters with humans occurs at a low frequency (4.6–5.9%; Parker, 1971; Lambert and Ferguson, 1985). Thus, the general absence of blood-squirting associated with handling by humans did not force rejection of a potential for blood-squirting.

In fact, of the 13 species recognized in the genus *Phrynosoma* (Montanucci, 1987; Zamudio et al., 1997; Zamudio and Parra-Olea, 2000), six are documented to squirt blood: *Phrynosoma asio* (Alvarez del Toro, 1960), *Phrynosoma cornutum* (Hurter, 1911; Winton, 1914; Lambert and Ferguson, 1985), *Phrynosoma coronatum* (Van Denburgh, 1922; Klauber, 1939; Burlson, 1942), *Phrynosoma orbiculare* (Ruthling, 1919; Cuesta Terron, 1932; Ditmars, 1951), *Phrynosoma solare* (Cutter, 1959; Parker, 1971), and *Phrynosoma taurus* (Ruthling, 1919). Of the species not reported to squirt blood, two are poorly studied forms endemic to Mexico, *Phrynosoma braconnieri* and *Phrynosoma ditmarsi,* but five are relatively well-known species from the United States and Mexico: *Phrynosoma douglasii, Phrynosoma hernandesi, Phrynosoma mcallii, Phrynosoma modestum,* and *Phrynosoma platyrhinos.*

Here, we use field capture records of herpetologists and encounters with a dog "predator" (Middendorf and Sherbrooke, 1992) to evaluate blood-squirting behavior in five species for which such behavior has not been reported. We use these data to modify the hypothesis that all species normally employ blood-squirting and then use a published phylogeny (1) to infer the evolutionary history of blood-squirting and (2) to predict the blood-squirting ability of species for which data are not available. Finally, in *P. cornutum,* we examine the effects of age, sex, left versus right eye, and body mass on squirt frequency and mass of blood expelled.

MATERIALS AND METHODS

Species tested for blood-squirting in dog trials.—Eleven adult *P. mcallii* (2 females, 9 males; SVL 62–74 mm, mean = 67.9 mm; mass 11–16 g, mean = 12.8 g) were captured in the field on the Barry M. Goldwater, Marine Corps Air Station, in Yuma, Arizona, on 23 May 1994. Nine lizards were collected and tested in the morning and two in the late afternoon. Because of the conservation status of this species in Arizona, 3-min dog trials were conducted at the site of capture inside the confines of a cardboard box (29 × 45 × 10 cm), the bottom of which was covered by sand. Lizards were released immediately following the trials. An adult approximately 10-year-old female chocolate Laborador retriever was encouraged to pursue and mouth capture the lizards within the box. With this dog, no barking or pawing occurred.

Ten adult *P. modestum* (1 female, 9 males; SVL 43–66 mm, mean = 52.9 mm; mass 5.4–13.4 g, mean = 9.4 g) were collected between 18 May and 22 June 1991 in Hidalgo County, New Mexico. They were housed outdoors in a 1.5-m diameter circular, top-screened fiberglass enclosure at the Southwestern Research Station, Portal, Arizona. Dog trials were conducted on 2 July 1991 between 0740 and 0810 h MST (air temperature 26.7–28.4 C), in Playas, Hidalgo County, New Mexico. The dog, a four-year-old female yellow Labrador retriever ("Dusty"), had previous experience in trial procedures, which included barking and pawing. Trials were conducted on the ground in an open area; lizard escape was restricted by the experimenters (Middendorf and Sherbrooke, 1992).

Two adult *P. platyrhinos* (SVL 72 and 83 mm, mass 12.4 and 16.8 g) were collected in late July 1994 in Yuma County and Maricopa County, Arizona. They were tested for squirting response in 3-min dog trials on the morning of 15 August 1994 in Playas, New Mexico. In 1995, 13 adult *P. platyrhinos* (7 females, 6 males; SVL 73–90 mm, mean = 78.7 mm; mass 15.8–26.8 g, mean = 19.8 g) were collected 27–30 April near the Mohawk Dunes, Yuma County, Arizona. These lizards were tested on 2 May 1995 in 3-min dog trials run between 0940 and 1035 h MST at air temperatures between 23 and 26 C in Playas, New Mexico. In both sets of trials "Dusty" was the trial dog, again in an open area.

One adult female *P. hernandesi* (SVL 72 mm; mass 27.5 g) was collected 7 June 1988 and tested with a young shepard-mix dog at 0935 h MST on 12 June 1988 at a San Simon Valley ranch in New Mexico. The lizard was released on the ground for pursuit by the dog. Three juvenile *P. hernandesi* (SVL 52, 57, 62 mm; mass 9.6, 10.0, 12.7 g, respectively) were collected 11–14 July 1988 and tested in dog trials (0800–0830 h MST; 23.8–25.0 C) at Rodeo, New Mexico, on 18 July 1988. Two adult female *P. hernandesi* (SVL 65 and 93 mm; mass 16.8 and 42.3 g) collected 26 July and 16 August 1990 were used in 3-min dog trials on 24 August 1990 at the Southwestern Research Station, Portal, Arizona. Ambient temperature was 27 C, time 1118–1135 h MST, and animals were in shade on open ground. In the two latter sets of trials, Dusty was the trial dog. All lizards were from the Chiricahua Mountains, Cochise County, Arizona.

Three adult *P. ditmarsi* that had been in captivity for an extended period were borrowed from R. R. Montanucci to test for blood-squirting. There were two females, SVL 72 and 74 mm, mass 28.7 and 30.0 g, and one male, SVL 71 mm, mass 18.6 g. Lizards were tested in 5-min dog trials on 13 November 1992, 1300–1320 h MST, in Playas, New Mexico, and Dusty was the trial dog.

Herpetologists with wide experience collecting horned lizard species for which blood-squirting had not been reported were surveyed as to their blood-squirting encounters with horned lizards. Numbers encountered, based on their field notes, were recorded as negative data when researchers clearly recalled never having been squirted by the species. In the few instances when squirting did occur, the circumstances and results are reported. Only first encounters with each individual were counted, not recaptures (unless indicated).

Variability in blood-squirting by Phrynosoma cornutum.—Forty adult Texas horned lizards, *P. cornutum* (SVL 71–98 mm, mean = 83.8 mm; mass 20.4–54.8 g, mean = 32.1 g; 5 females, 35 males), were collected in Cochise County, Arizona, and Hidalgo County, New Mexico, between 3 May and 7 June, 1991. Each lizard was measured, weighed, sexed, and individually numbered on the ventrum before being housed outdoors in a 5.5 × 4.6 × 2.2 m screened, retaining cage at the Southwestern Research Station, in the Chiricahua Mountains, near Portal, Arizona. Prior to testing, seed-harvester ants (*Pogonomyrmex* spp.) were provided ad libitum as food, and water was supplied by natural rainfall and hose sprinkler, about weekly, for "rain-harvesting" (Sherbrooke, 1990b). Once the trials began, trial animals were not fed or watered until they ceased to respond, after which they were returned to the retaining cage. Lizards were weighed before the first trial and following each trial. After the last trial, all lizards were sprinkled with water and fed. Trials were conducted from approximately 0700–1115 h MST in a grassy arena in Playas, New Mexico, between 19 and 25 June 1991. Fully shaded, air temperatures (2 cm above substrate) ranged between 22.4 and 33.6 C. Dusty was used for these trial procedures. Taped verbal recordings and written notes were made of the lizards' behavior during the trials.

To limit exposure of the dog to the lizard's horns and blood, we allowed the dog and lizards to interact a maximum of two minutes. Subsequently, or when eyelid swelling ("eye puffiness," Middendorf and Sherbrooke, 1992) or blood-squirting was first observed, the lizard was picked up by one of the experimenters, and for a period of 1.5 min the eyelids were stroked with two index fingers, simultaneously and repeatedly touching right and left eyelids. We collected blood squirted during the human manipulation by holding the lizard within the open end of a heparinized (Sigma heparin H-1551; 100 units/ml) clear plastic bag. Records were made of frequency of squirts from each eye. Bags were immediately closed and placed in a cooler on ice. Prior to use we had individually numbered and weighed the plastic bags (279 × 330 mm and 0.8 mil thick). External surfaces of the bags were dried of water before reweighing. The mass of blood squirted was determined by the change in mass of the bag and its contents before and after use in a trial.

Not all lizards squirted blood in response to the dog encounters (barking, pawing, gentle biting, nibbling, and picking up and tossing of the lizard) or to the subsequent manipulation by the experimenter. All lizards were tested on the first two days. Lizards that did not respond on either day were not run in subsequent daily trials. During successive trials, lizards that did not respond in any single trial were eliminated from further trials. Trials were terminated after the seventh day when only two animals squirted minor amounts of blood.

Five juvenile *Phrynosoma cornutum* were collected between 21 May and 2 June 1991 in the same areas as the adults. Their snout–vent lengths were 36–46 mm, mean = 39.2 mm, and their mass (19 June 1991) was 2.9–10.2 g, mean = 4.9 g. Juveniles were tested on 20 June 1991 under similar conditions as adults, in Playas, New Mexico. Dog trials were run 0855–0915 h MST, 28.6–30.2 C.

Statistical analyses on numbers of lizards squirting blood in the different treatments were preformed with a statistical package for social sciences (SPSS 8.0.0, 1997, Chicago, IL, unpubl.). Significance levels were determined by comparing *P*-values to an alpha level of 0.05. Means are reported with ± 1 standard deviation.

Results

Species tested for blood-squirting in dog trials.—In 11 dog trials with *P. mcallii*, all lizards failed to squirt blood (0%) or exhibit eye puffiness because of engorgement of the ocular sinuses. Lizards kept their eyes open throughout the trials.

In the 10 dog trials with *P. modestum*, no lizards squirted blood (0%) in response to the dog, nor did any lizard exhibit eye puffiness. None of the lizards raised its tail (Middendorf and Sherbrooke, 1992), nor oriented its head spines upward (Sherbrooke, 1987), but two animals opened their mouths and protruded the tongue.

In *P. platyrhinos*, neither blood-squirting (0%) nor eye puffiness in the ocular sinus area was noted in the two 1994 dog trials. One lizard exhibited several tail-raises and open-mouth displays (Sherbrooke, 1991). In the 13 *P. platyrhinos* dog trials in 1995, again no lizard squirted blood (0%) or increased ocular sinus blood pressure enough to exhibit eye puffiness. Several lizards opened their mouth and protruded their tongue or raised their tail.

Of the three juvenile *P. hernandesi*, one (the largest) squirted blood after 2.5 min, and two failed to squirt blood (7.7 min; 8.8 min). All three juveniles exhibited predator-defense behaviors (puffed-up body, open-mouth lunges/attacks). In the one adult-female lizard trial with a dog in 1988, the lizard squirted nine times. In the two 1990 dog trials with adult *P. hernandesi*, one of the lizards squirted blood in response to the dog's actions, and the other did not. Thus, in the total of six dog trials, three lizards (50%) squirted blood.

None of the three *P. ditmarsi* squirted blood in dog trials (0%), but they did exhibit open mouth threats. Eyes were maintained open during the dog encounters, but immediately afterward, when picked up by the experimenter and the back was vigorously rubbed, each lizard exhibited eye puffiness indicating increased blood pressure in the circumocular sinuses.

Human encounters with Phrynosoma mcallii, Phrynosoma modestum, and Phrynosoma platyrhinos.—In the case of *P. mcallii,* one of us (WCS) has never observed blood-squirting in handling 25 captured individuals from Yuma County, Arizona, and Imperial County, California. Other herpetologists questioned about their experiences reported similar negative results in handling field-caught *P. mcallii*: W. Hodges (n = 34), W. W. Mayhew (n > 400), P. A. Medica and F. B. Turner (n = 63), B. Morrill (n = 231), A. Muth and M. Fisher (n = 394), K. Young and A. Young (n = 450). Therefore, in over 1500 field encounters with and handlings of *P. mcallii,* blood-squirting has never been observed.

One of us (WCS) has collected (or received with information from collaborators) and cataloged 308 *P. modestum* from Hidalgo County, New Mexico, and Cochise County and Graham County, Arizona, between 1976 and 1999. Blood-squirting was never observed as a reaction to human collection or to subsequent holding in cloth bags or to other handling in captivity. Nevertheless, three cases of blood-squirting in *P. modestum* were verbally reported to us and evaluated as to the circumstances and likelihood of being blood-squirting events. One report was a road-collected *P. modestum* that resulted in blood on the thumb of the collector. Because of the presence of ants at the lizard, and the lizard's subsequent behavior, it apparently was injured before being encountered. Therefore, the blood was a result determined to be from that injury not from blood-squirting in response to capture. Another road-captured animal in Texas, during the evening, apparently squirted blood (C. R. Harrison, pers. comm.), and an afternoon field-encountered *P. modestum* in New Mexico squirted blood "all over a white T-shirt," (P. A. Medica, pers. comm.). Unfortunately voucher specimens to confirm identity are not available.

In the case of *P. platyrhinos,* one of us (WCS) has never observed blood-squirting in handling 30 individuals from Maricopa County and Yuma County, Arizona, and Imperial County and San Bernardino County, California. Other herpetologists surveyed about their experiences reported similar negative results in handling over 1000 field-caught *P. platyrhinos*: W. Hodges (n = 22), W. B. Jennings (n = 50), W. W. Mayhew (n > 100), P. A. Medica and F. B. Turner (n = 345), B. Morrell (n = 27), A. Muth and M. Fisher (n = 42), E. R. Pianka and W. S. Parker (n = 364), K. Young and A. Young (n = 130). But, in a still larger sample of 2569 field-collected *P. platyrhinos,* one animal was reported to have squirted blood (three small droplets) in response to human handling, and one squirted blood postenvenomation by a *Crotalus viridis lutosus* bite (S. Ferrand; pers. comm).

In addition, a female *P. platyrhinos* (SVL 70 mm, mass 78 g) from the Mohawk Dunes, Yuma County, Arizona, squirted blood on 1 May 1998 (B. Pollock, pers. comm.). The lizard was placed in a bucket with several other lizards (*Dipsosaurus dorsalis* and *Cnemidophorus* sp.) and exhibited "violent" reactions to the other lizards, puffing up, lunging, and biting. When the lizard was picked up, blood appeared on Pollock's hand, apparently a result of squirting by the desert horned lizard. Subsequently, this individual remained aggressive in captivity and bit Pollock on several occasions but did not squirt blood when introduced to her dog, a golden retriever. Thus, in over 3500 encounters with humans, only two credible cases of blood-squirting are known for *P. platyrhinos.*

Variability in blood-squirting by Phrynosoma cornutum.—In 151 trials of blood-squirting by 40 Texas horned lizards, a total of 1085 squirts was elicited. Although both eyes were stroked an equivalent number of times with index fingers, for animals that squirted at least once, we observed more squirts from the left than from the right eye (L: mean = 6.3 ± 7.7; R: mean = 3.6 ± 4.1; t = 3.35; df = 108; P = 0.001). The number of trials in which squirting was restricted to the left eye alone was greater than to the right eye (L: mean = 7.9 ± 10.3, n = 33; R: mean = 4.6 ± 3.7, n = 20). Even for those individuals squirting from both eyes, which were seen in about half of the trials (57 of 109 trials), the number of squirts from the left eye again exceeded those from the right eye (L: mean = 7.7 ± 5.9; R: mean = 5.4 ± 4.2, n = 56). We suspect that some of these differences may have been an artifact of experimenter handedness.

Frequency of squirting by individual lizards varied from zero (six lizards) to 86 squirts over the trials. Eighty-five percent of all animals squirted, and 82% squirted in more than one trial (Table 1). Two lizards squirted repeatedly over the entire seven-day trial period. As a function of trial day, the frequency of squirting individuals (as opposed to nonsquirters) ranged between 33.3 and 100% (Table 1), with no apparent trend during the seven-day trial period. Numbers of squirt responses per individual on any single trial day over the seven-day trial period ranged greatly (mean = 27.0 ± 26.0).

For the 109 trials in which animals squirted

TABLE 1.

Trial Day	1	2	3	4	5	6	7
$\bar{x}$ squirts	11.6 ± 8.7	13.1 ± 10.8	8.0 ± 7.4	5.7 ± 4.4	7.9 ± 10.3	6.0 ± 4.1	3.0 ± 1.4
$\bar{x}$ mass of squirters (g)	33.5 ± 11.2	34.1 ± 9.4	36.7 ± 9.2	37.5 ± 8.8	39.8 ± 8.7	40.1 ± 10.1	33.3 ± 0.5
Animals squirting (%)	85.0	67.5	63.6	73.3	72.7	100	33.3
Sample size (n)	40	40	33	15	11	6	6

(71% of all trials), the average response of an individual lizard was 10.0 ± 8.9 squirts; however, one individual squirted 54 times from one eye. Larger animals were more likely than smaller ones to squirt blood repeatedly over consecutive trial days (Fig. 1; $r^2 = 0.07$; $P = 0.11$). When data for a single, extraordinarily large, gravid female (69.8 g) that squirted only on the first trial day (six squirts) was removed from the analysis, both the fit and significance greatly increase ($r^2 = 0.23$; $P = 0.04$). Similarly, a near-significant trend for larger animals to squirt more often than smaller lizards (Fig. 2; $r^2 = 0.09$; $P > 0.08$) becomes significant ($r^2 = 0.28$; $P < 0.001$) when that same large female was removed from the analysis.

Gender differences were noted (females $n = 5$, mean mass 50.2 ± 14.3 g, mean squirts 37.4 ± 29.0, mean consecutive number of days in which the lizards squirted 2.8 ± 2.9; males $n = 35$, mean mass 30.5 ± 7.4 g, mean total number of squirts 25.7 ± 26.0, mean days 2.7 ± 2.1), but may well be influenced by that same large female (data not analyzed statistically because of small female sample size). The four, considerably smaller (presumably nongravid) females (mean = 45.5 ± 9.5 g) used in the study squirted for more than a single day (mean = 3.25 ± 1.6 days; range 2–6) and more frequently (mean = 45.5 ± 23.0 squirts; range 21–73) than the large gravid female. In contrast, body mass of males squirting at minimal levels (≤ 6 times) was less than that of males squirting more often (mean = 27.8 ± 6.1 g; $n = 11$ vs mean = 31.7 ± 7.8 g; $n = 24$).

The number of squirts decreased significantly as a function of squirting over multiple days (Fig. 3; $r^2 = 0.20$; $P < 0.05$). Cumulative mass loss for individual lizards increased as a function of the number of squirts ($r^2 = 0.02$; $P < 0.05$), no doubt attributable to the direct function of both the number of days in which lizards squirted blood (Fig. 3; $r^2 = 0.81$; $P < 0.001$) and the total squirt number ($r^2 = 0.70$; $P < 0.001$).

An average squirt weighed 0.025 g. The total mass of squirted blood is directly related to the number of squirts given during a trial (Fig. 4; $r^2 = 0.66$; $P < 0.001$). Average blood mass squirted by individuals ranged between 0.01 and 1.5 g (mean = 0.3 ± 0.3 g; $n = 99$; in 10 of the 109 trials in which squirting was observed blood mass was not measurable; for example, single drops weighing < 0.01 g) and cumulative blood-mass squirted for all animals varied between zero (for those lizards not squirting at all) and 2.8 g (mean = 0.7 ± 0.8 g; $n = 40$). Average

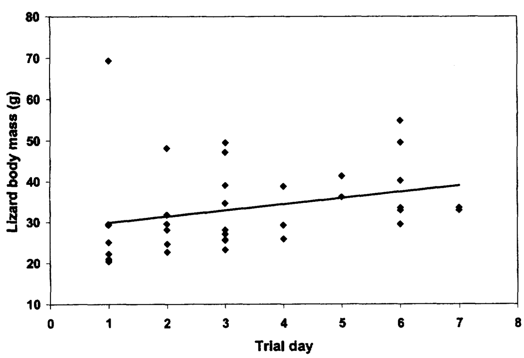

Fig. 1. Regression of lizard (*Phrynosoma cornutum*) body mass on trial day ($r^2 = 0.07$, $P = 0.11$ as illustrated; $r^2 = 0.23$, $P = 0.004$ with outlier omitted).

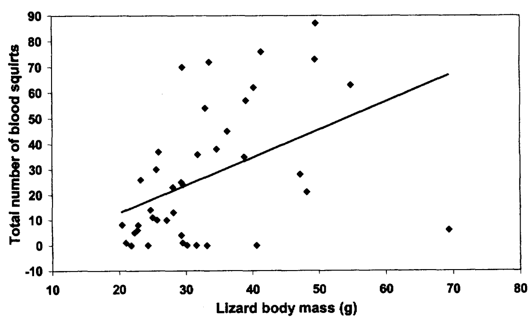

Fig. 2. Influence of lizard (*Phrynosoma cornutum*) body mass on the total number of blood squirts recorded ($r^2 = 0.09$, $P > 0.08$ as illustrated; $r^2 = 0.28$, $P = 0.001$ with outlier omitted).

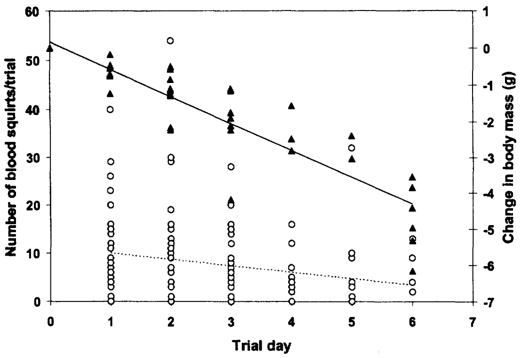

Fig. 3. Regression of number of lizard (*Phrynosoma cornutum*) blood squirts per trial (open circles, dashed line; $r^2 = 0.20$, $P < 0.05$) and loss of mass (g) (solid triangles, solid line; $r^2 = 0.81$, $P < 0.001$) on trial day.

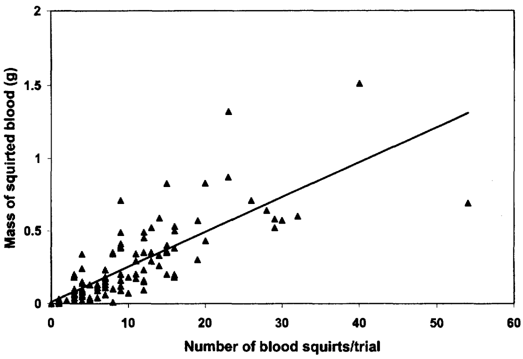

Fig. 4. Mass of squirted blood (from the eye orbits of *Phrynosoma cornutum*) as a function of number of blood squirts elicited during trials in which blood was squirted ($r^2 = 0.66$; $P < 0.001$).

percent individual loss of body mass attributable to squirted blood during a single trial ranged between 0.03 and 3.2% (mean = 0.8 ± 0.7%; $n = 99$), whereas cumulative percent loss of body mass over all trials ranged from zero (individuals not squirting) to 6.8% (mean = 2.0 ± 2.0%; $n = 40$).

Dog trials with juvenile *P. cornutum* resulted in blood-squirting by three of the five lizards (60%; the smallest and the largest individuals did not squirt blood), verifying that juvenile animals with small mass are capable of this behavior.

Discussion

In only one of the five species evaluated with dog trials was there a clear blood-squirting response; three of six *P. hernandesi* individuals squirted blood. Although we have not found reports in the literature of blood-squirting for *P. hernandesi,* we have received several verbal and written communications of blood-squirting events during encounters with humans and dogs. These observations together with the positive dog-trial results demonstrate that this polytypic species (Stebbins, 1954), at least in certain parts of its range, is capable of squirting blood. Despite this conclusion, W. S. Parker (pers. com.) failed to elicit blood-squirting while capturing 47 *P. hernandesi* in Utah (Pianka and Parker, 1975), and C. Guyer (pers. comm.) also failed to elicit it in 162 *P. hernandesi* in southeastern Idaho (Guyer, 1991).

None of the three *P. ditmarsi* tested in dog trials exhibited blood-squirting. But immediately subsequent to the dog trials, "rough" human handling of the lizards resulted in swelling of the orbital sinuses, indicating constriction of sphincter muscles associated with cranial veins (Bruner, 1907), which is a key physiological prerequisite for blood-squirting (Middendorf and Sherbrooke, 1992). These preliminary results, phylogenetic considerations discussed below, and unpublished positive results of dog trials in the field (W. Hodges, pers. comm.) lead us to the conclusion that *P. ditmarsi* is capable of squirting blood.

Larger numbers of lizards were used in dog trials with three species, *P. mcallii* ($n = 11$), *P. modestum* ($n = 10$), and *P. platyrhinos* ($n = 15$). No instances of blood-squirting or eye puffiness were noted in any of the trials. These results, particularly as they dramatically contrast to our earlier dog-trial results with *P. cornutum* (Middendorf and Sherbrooke, 1992), suggest that these three species do not squirt blood when confronted by a canid predator. Because blood-squirting responses to human encounters are much lower (*P. cornutum* 5.9% and *P. solare* 4.6%) than those elicited in dog trials (70–100%), negative responses to human encounters are difficult to evaluate. Further, there is no a priori reason why species should be similar to each other in the frequency of this response. However, the large number of such encounters with humans without blood-squirting recorded (*P. mcallii* $n > 1500$; *P. modestum* $n > 300$; *P. platyrhinos* $n > 3500$) are supportive of the negative results obtained in the dog trials. The rare credible instances of blood-squirting reported to us for *P. platyrhinos* and *P. modestum* suggest that these species retain some ability to squirt blood. However, these cases represent four positive out of 5527 negative blood-squirting encounters reported by field collectors. Conservatively, the blood-squirting frequency to human encounters for the *P. mcallii-modestum-platyrhinos* group is 0.07%, almost two orders of

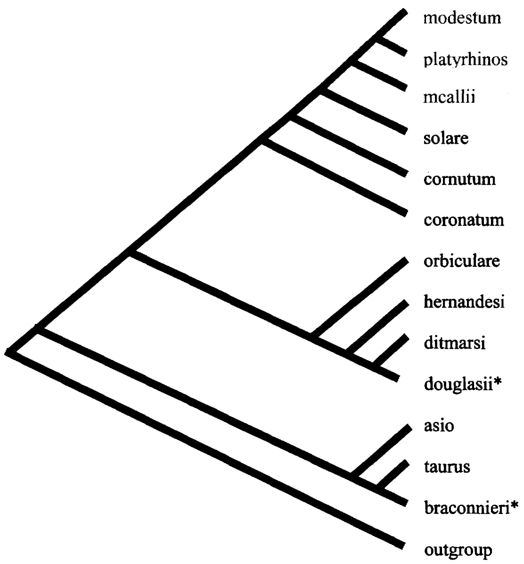

Fig. 5. Blood-squirting and nonblood-squirting species of *Phrynosoma* adapted to a cladogram of the genus (Zamudio and Parra-Olea, 2000, as adapted from Montanucci, 1987, and Zamudio et al., 1997). The *Phrynosoma mcallii-modestum-platyrhinos* clade, in gray, is hypothesized to have lost blood-squirting as an antipredator defense. The blood-squirting status of two species remains unresolved: *Phrynosoma douglasii** may or may not have lost this mechanism independently, and *Phrynosoma braconnieri** is predicted to be a blood-squirting species. All other species in the genus are known to squirt blood, and no outgroup members squirt blood from the ocular sinus.

magnitude below the reported frequency for *P. cornutum* (5.9%; Lambert and Ferguson, 1985) and *P. solare* (4.6%; Parker, 1971). The blood-squirting response rates in dog trials for species tested are: *P. mcallii* (0%), *P. modestum* (0%), *P. platyrhinos* (0%), versus *P. cornutum* (70–100%), *P. hernandesi* (50%), *P. solare* (60%; three of five lizards in dog trials; WCS, pers. obs.). From an evolutionary perspective, it should be noted that unusual stress situations have occasionally induced ocular bleeding or autohemorrhaging in other genera of lizards (noosing by humans: *Urosaurus ornatus,* Mahrt, 1996; *Sceloporus jarrovii,* Sherbrooke, 2000) and in snakes (Smith et al., 1993).

The genus *Phrynosoma* is monophyletic (Fig. 5) and is most closely related to sand lizards (*Uma, Callisaurus, Cophosaurus,* and *Holbrookia*; Frost and Etheridge, 1989; Reeder, 1995; Macey et al., 1997). Within the genus *Phrynosoma,* a number of somewhat divergent phylogenetic interpretations on the relationships of species have been suggested based on morphological (Reeve, 1952; Presch, 1969; Montanucci, 1987) and molecular (Zamudio et al., 1997; Zamudio and Parra-Olea, 2000; Reeder and Montanucci, 2001) data. Nevertheless, these diverse phylogenetic treatments consistently group three species, *P. mcallii, P. modestum,* and *P. platyrhinos,* together, although sometimes with *P. solare* in various sister-species groups. Because *P. solare* exhibits blood-squirting (Parker, 1971), this behavior might be considered the ancestral condition for the four-species group; alternatively, the behavior might have been absent in the ancestor of these four species, becoming reestablished in *P. solare.*

Two species remain for which there are no reports of blood-squirting nor evaluations of this response by means of dog trials: *P. braconnieri* and *P. douglasii. Phrynosoma braconnieri* has a very restricted distribution in Mexico, and its behavior is poorly known. *Phrynosoma braconnieri* is consistently linked, phylogenetically, with *P. asio* and *P. taurus* (Fig. 5), both species that squirt blood, and sometimes with *P. ditmarsi,* which we believe squirts blood (see above). Based solely on phylogenetic considerations, we suggest that this species will be found to exhibit blood-squirting behavior in certain predator/prey encounters.

Phrynosoma douglasii has recently been redesignated as a species distinct from *P. hernandesi* (Zamudio, 1996; Zamudio et al., 1997). Aspects of its behavior and ecology have been studied by Zamudio (1996), who reports (pers. comm., unpubl. data) no blood-squirting in 400 individuals (3000 recaptures), suggesting that the species may not squirt blood. But, she noted occasional eye swelling (pers. comm.) and marked differences from other short-horned lizards in predator avoidance behaviors (Zamudio et al., 1997). The phylogenetic position of *P. douglasii* (Zamudio and Parra-Olea, 2000) indicates a close relationship and evolutionary history with species of horned lizards that are known blood-squirting species (Fig. 5). This might suggest that, with appropriate testing for antipredator responses, this species will be shown to exhibit a blood-squirting response. Nevertheless, the lack of blood-squirting responses to humans may indicate an autapomorphic condition derived independently from the *P. mcallii-modestum-platyrhinos* species clade. We do not feel that the diminutive size of lizards in this species precludes it exhibiting blood-squirting, because small juvenile *P. cornutum* and *P. hernandesi* responded in our dog trials. *Phrynosoma douglasii* needs to be tested with dog or other predator trials before concluding that it does not squirt blood.

Nevertheless, size is of significant consequence for horned lizards in the outcome of predator-prey encounters (Sherbrooke, 1990a, 1991) and may have significance in the evolution of blood-squirting in *Phrynosoma.* Smaller species have a more diverse array of predators than do larger species, a feature that may be associated with our observation that, if two lineages (Fig. 5) have effectively lost the blood-squirting defense, they include the two smallest species in the genus, *P. modestum* and *P. douglasii.*

Based on Thorson's (1968) estimate that *Iguana* blood constituted 6% of total body mass, we calculated individual blood losses via squirting by *P. cornutum* in a single trial to vary between 0.5 and 53% of total body blood (mean = 13.1 ± 11.0%; n = 99). Over the course of all trials, the cumulative blood loss for individuals ranged from zero to 113% (mean = 32.5 ± 32.5%; n = 40). With such potential blood losses we hypothesize that the lizards must have rehydrated the circulatory fluid via depletion of water from other tissues (blood squirted on the seventh day was "watery," pinkish rather than red in color; pers. obs.). Average percent individual loss of body mass attributable to squirted blood was highly variable, 0.03–3.2% in a single trial and as high as 6.8% over repetitive trials.

The frequency of squirts exhibited in individual trials and the daily repeated encounters of stimulation for blood-squirting in the experiment were undoubtedly more physiologically demanding than we believe to be the case in natural predator/prey encounters (pers. obs., kit fox trials). Nevertheless, the experimental blood-loss data suggest that behavioral mechanisms limiting blood loss during repeated stimulation of this response are weak or nonexistent (e.g., continuation of blood-squirting is only limited by physiological parameters).

Without the use of a blood-squirting defense and its presumed delivery of noxious chemicals (which may be contained in circulating as well as squirted blood; Middendorf et al., 2001), a horned lizard may have little chance of surviving an encounter with a canid, because both coyotes and kit foxes (pers. obs.) eat *P. cornutum.* With a blood-squirting defense a lizard's survival chances increase, probably substantially (pers. obs., kit fox trials). The physiological costs of the defense, in terms of blood loss, can be low or high. The success of this defense behavior depends on factors such as the efficacy with which squirted blood is delivered to membranes in the mouth of the predator (W. C. Sherbrooke and J. R. Mason, unpubl. data) and the experience and hunger state of the individual canid.

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